



A Rapid, High Frequency Regeneration of *Justicia gendarussa* Burm.f.

B. Janarthanam^a, B. Gayathri^c and E. Sumathi^{b*}

^aPlant Tissue Culture Lab, P.G & Research Department of Botany, Pachaiyappa's College, University of Madras, Chennai 600 030, Tamil Nadu, India. ^bDepartment of Biotechnology, D.G. Vaishnav College, University of Madras, Arumbakkam, Chennai - 600106, Tamil Nadu, India. and ^cFood Technology, Centre for Biotechnology, Anna University, Chennai -600 025

Abstract

An efficient plant regeneration protocol was developed for *Justicia gendarussa* Burm.f. (Acanthaceae), an important medicinal shrub. Nodal segments grown on Murashige and Skoog (MS) medium containing 1.0 mg/l Benzyl adenine (BAP) with 10 % coconut milk showed better growth response and produced 10.5 ± 0.6 shoots per explant with an average length of 4.4 ± 0.3 cm after 35 days. Rooting of shoots was achieved on growth regulator free half strength MS medium produced 5.3 ± 0.25 cm roots with an average height of 4.8 ± 0.2 cm after 30 days. The rooted plantlets were transferred for hardening, 80 % of plants were successfully established in the field.

Key words: *Justicia gendarussa*, Nodal segments, Regeneration, Coconut milk.

Introduction

Justicia gendarussa Burm. F. (Acanthaceae) is an erect, branched, smooth undershrub, 0.8 - 1.5 meters in height with long leaves (7 to 14 cm) having acute tips; small flowers, terminal pinkish spikes with purple spots. It is found throughout India and in Asian countries like Malaysia, Indonesia and Sri Lanka (Rantnasooriya *et al.*, 2007). The extracts of roots and leaves are used as important ingredient of many ayurvedic preparations (Varier, 1994). The plant is used as traditional medicine for chronic rheumatism, inflammations, bronchitis, head ache, arthritis, facial paralysis, internal hemorrhages, vaginal discharges, dyspepsia, eye disease and common fever (Chopra *et al.*, 1986). *J. gendarussa* has been reported to have higher mineral profiles in leaves (Ca, Mg & Zn) (Corlett *et al.*, 2002) and possess antivenom properties (Maiti and Mishra, 2000). The seeds of *J. gendarussa* show a very low germination percentage (Mrunthunjaya and Hukkeri, 2007). Earlier *In vitro* culture of *J. gendarussa* has been attempted through organogenesis (Agastian *et al.*, 2006). The present study aims to develop an alternative simple, rapid, economical, and high frequency of plantlet regeneration through nodal explants for large scale propagation.

Material and Methods

Plant material

Healthy plants of *Justicia gendarussa* collected from Surapet village near Chennai, Tamil Nadu, India and were raised in pots containing soil and farm yard manure (1:1) under greenhouse conditions at Department of Biotechnology, D.G. Vaishnav College, Chennai- 600 106.

Explant preparation and shoot regeneration

Nodal segments were cut from processed for aseptic culture. Explants were cleaned thoroughly under running tap water for 20 minutes; washed with a solution of Tween 20 (2 drops in 100 ml of water) for 1 min, and again washed with sterile distilled water. The cleaned explants were finally treated with HgCl₂ (0.1% w/v) for 4-5 minutes under aseptic conditions and washed 5 times with sterile distilled water to remove traces of HgCl₂. The process of explant preparation was done under / in the Laminar Flow cabinet/ Chamber.

After surface sterilization, explants were trimmed to 0.8 - 1.0 cm and inoculated on MS basal medium (Murashige and Skoog, 1962) supplemented with different concentrations of BAP (0.25, 0.5, 1.0 and 2.0 mg/l), Kinetin (KN) (0.25, 0.5, 1.0 and 2.0 mg/l) with Coconut milk (10.0 %) for shoot mul-

* Corresponding author: E-mail: sumathiethiraj@yahoo.com

tiplication. The proliferated shootlets 4.5-5.0 cm length was transferred to half strength MS medium supplemented with no growth regulator for root development. Root number and length were recorded after 30 days in culture. Well developed plantlets were rinsed thoroughly with sterile water to remove residuals and potted with a mixture of red soil, vermiculite and farm yard manure (1:1:1), covered with moistened polythene bags for hardening. After 15 days, the fully acclimatized plantlets were transplanted to pots (6 cm dia).

Culture medium and conditions

MS basal medium supplemented with 3% (w/v) sucrose was used for all *in vitro* culture studies. The pH of the medium was adjusted to 5.6 ± 0.2 prior to adding 0.9 % (w/v) agar, and autoclaved at 121°C at 1.06 kg cm⁻² for 15 min. Cultures were maintained at 25°C under 16h photoperiod with a photosynthetic photon flux density (PPFD) of 50 μmol m⁻²s⁻² provided by cool white fluorescent lamps (Phillips, India) and with 60 - 65 % relative humidity. The plant growth regulators were filter-sterilized using 0.2 μm filter (Minisart®, Sartorius) prior to addition to culture media.

Results and Discussion

Multiple shoots developed from nodal explants cultured on MS medium supplemented with BAP (0.25 - 2.0 mg/l), KN (0.25 - 2.0 mg/l) and fortified with various concentrations of

coconut milk (5.0, 10.0, and 15.0 %). Initiation of multiple shoots in most of the treatments was observed within 3 weeks of culture. High number of multiple shoots developed in MS medium containing BAP 1.0 mg/l with 10% coconut milk (CoM), 80% response and produced 10.5 ± 0.6 shoots per explant with an average length of 4.4 ± 0.3 cm after 35 days (Table I; Fig. 1A, B & C). Whereas higher concentration of BAP (2.0 mg/l) containing medium did not significantly induce the number of shoots per explants (Table I). BAP has been considered to be one of the most active cytokinins in organogenic differentiation in plant tissue culture (Fracaro and Echeverrigaray, 2004; Gururaj *et al.*, 2007; Baskaran and Jayabalan, 2005). The use of BAP with 10 % coconut milk (CoM) in our study, the development of multiple shoot production is superior to the earlier observation on using MS medium supplemented with higher concentration of BA (3.0 mg/l) were recorded 4.3 shoots per explant (Bushrabi *et al.*, 2008). A combination of other growth substances including Kinetin (KN) + CoM was not effective (Table I).

The regenerated shoots with 4-5 leaves were rooted on the growth regulator free half strength MS medium. The first roots appeared after 2 weeks of culture, and after 25 days, the root system was well developed (fig. 1D & E). The percentage of rooting was 100% and 5.3 ± 0.25 roots per shoot with an average length of 4.8 ± 0.2 cm induced after 30 days

Table I: Effect of individual BAP or Kin with 10 % coconut milk in MS medium on *in vitro* shoot multiplication from nodal explants of *J. gendarussa* after 35 days of culture

Plant growth regulators (mg/l)		Shoot Induction%	Number of shoots per explant	Shoot length (cm)*
BAP	% of Coconut water added with medium			
0.1 mg/l	10%	46.8	3.4 ± 0.1	3.0 ± 0.6
0.25 mg/l	10%	55.9	4.0 ± 0.2	3.4 ± 0.2
0.5 mg/l	10%	80.0	10.5 ± 0.6	4.4 ± 0.3
1.0 mg/l	10%	51.8	3.7 ± 0.2	3.1 ± 0.8
2.0 mg/l	10%	39.8	2.9 ± 0.3	1.8 ± 0.2
KN				
0.1 mg/l	10%	-	-	-
0.25 mg/l	10 %	16.9	2.3 ± 0.1	0.8 ± 0.1
0.5 mg/l	10 %	29.6	3.4 ± 0.2	1.2 ± 0.1
1.0 mg/l	10 %	19.8	2.5 ± 0.1	1.1 ± 0.2
2.0 mg/l	10%	-	-	-

Results represent mean ± SD of three replicated experiments. Data were recorded after 35 days of culture.

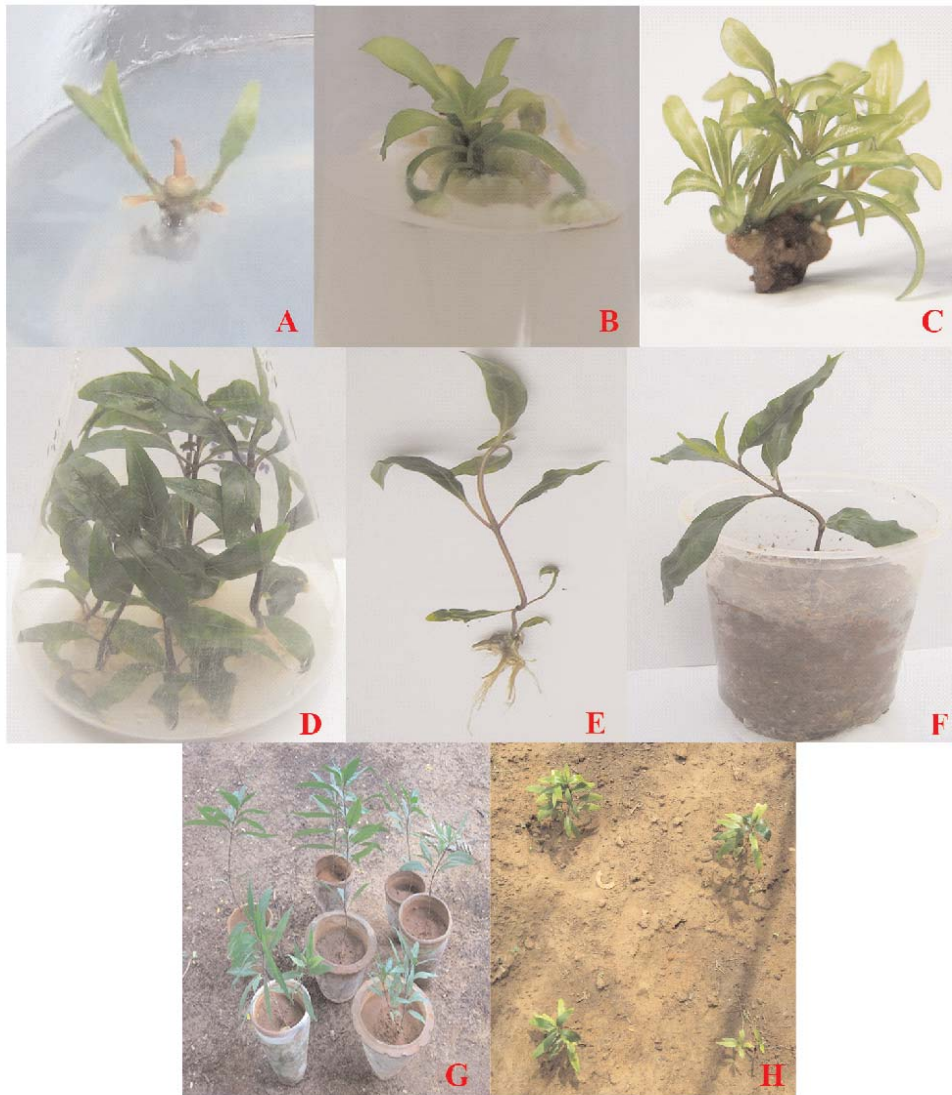


Fig. 1: Regeneration of multiple shoots from nodal explants of *Justicia gendarussa*

Stages in the micropropagation of *J. gendarussa*

- (A) Initiation of shoot from nodal explant after two weeks of culture.
- (B) Multiple shoots initiation after three weeks of culture on MS medium containing 1.0 mg/l BAP with 10% coconut milk
- (C) Proliferation of multiple shoots from nodal explant after 35 days of culture.
- (D) Rooted plantlet after 30 days of culture on growth regulator free ½ strength MS medium
- (E) A well established plant
- (F) Hardened *in vitro* plant successfully transplanted to the plastic cup
- (G) Acclimatized plantlets successfully transplanted to the pots
- (H) In field condition

old culture on root induction medium (Table II). The high number of roots per shoot produced on half strength growth regulator free medium in *J. gendarussa* with subsequent high survival rate.

Table II: Root formation from *in vitro* grown shoots of *J. gendarussa* after 30 days of culture

Medium	% response	Roots / shoot	Root length (cm)
½ MS	100.0 ± 0.0	5.3 ± 0.25	4.8 ± 0.2

Results represent mean ± SD of three replicated experiments. Data were recorded after 30 days of culture

Conclusion

One hundred percent plantlet survival was seen after hardening of the regenerated *J. gendarussa* in red soil, vermiculite and farmyard manure (1:1:1) for 3 wk. However, the rate decreased as some plants died over the next 4-10 wk after transfer to soil. It was observed that very gradual acclimatization of *in vitro* grown plants to the external environment is most essential for *J. gendarussa*. Eighty percent of the plants transferred to pots survived and resumed growth (Figure 1F, G & H).

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